MUTANT CSGG PORES

[0001] This application is a continuation of U.S. application Ser. No. 16/522,591, filed Jul. 25, 2019, which is a continuation of U.S. application Ser. No. 15/507,947, filed on Mar. 1, 2017, now U.S. Pat. No. 10,400,014, which is a national stage filing under 35 U.S.C. § 371 of PCT International Application No. PCT/EP2015/069965, which has an international filing date of Sep. 1, 2015, and claims foreign priority benefits under 35 U.S.C. § 119(a)-(d) or 35 U.S.C. § 365(b) of United Kingdom application number 1415455.3, filed Sep. 1, 2014, United Kingdom application number 1422079.2, filed Dec. 11, 2014, United Kingdom application number 1506489.2, filed Apr. 16, 2015, United Kingdom application number 1506754.9, filed Apr. 21, 2015, United Kingdom application number 1508287.8, filed May 14, 2015, United Kingdom application number 1511203.0, filed Jun. 25, 2015, and United Kingdom application number 1515240.8, filed Aug. 27, 2015. The contents of the aforementioned applications are herein incorporated by reference in their entireties.

FIELD OF THE INVENTION

[0002] The present invention relates to novel protein pores and their uses. In particular it relates to biological nanopores in nucleic acid sequencing applications, and molecular sensing.

[0003] The invention relates to mutant forms of CsgG. The invention also relates to analyte detection and characterisation using CsgG.

BACKGROUND OF THE INVENTION

[0004] Protein pores are membrane spanning polypeptides and complexes that form a channel in the membrane through which ions and certain molecules may pass. The minimum diameter of the channel is typically in the nanometre (10^{-9} metre) range hence giving certain of these polypeptides the name "nanopores".

[0005] Nanopores have great potential as biological sensors. When an electrical potential is applied across a membrane-bound nanopore, ions flow through the channel. This flow of ions can be measured as an electrical current. Suitable electrical measurement techniques using single channel recording equipment are described in, for example, WO 2000/28312 and D. Stoddart et al., Proc. Natl. Acad. Sci., 2010, 10 7702-7. Mufti-channel recording techniques are described, for example, hi WO 2009/077734.

[0006] A molecule translating though the pore, or binding in or near the pore acts to obstruct and thereby reduce the ion flow through the channel. The degree of reduction in ion flow, as measured by the reduction in electrical current, is indicative of the size of the obstruction within, or in the vicinity of, the pore. The measured electrical current can therefore be used as a measure of the size or degree of obstruction to the channel.

[0007] The changes in electrical current can be used to identify that a molecule, or part of a molecule, has bound at or near the pore (molecular sensing), or in certain systems, it can be used to determine the identity of a molecule that is present within the pore based on its size (nucleic acid sequencing).

[0008] The "Strand Sequencing" method is known for sequencing nucleic acids using biological nanopores. On passing a single polynucleotide strand through a nanopore, the bases on individual nucleotides are determined by the changes in measured electrical current as they pass transiently through the channel of the nanopore. This method offers significant time and cost savings over historic methods of nucleic acid sequencing.

[0009] Previously reported protein nanopores, such as the mutant MspA (Manrao et al., Nature Biotechnology, 2012, 30(4), 349-353) and alpha-hernolysin nanopores (Nat. Nanotechnol., 2009, 4(4), 265-70) have been used for nucleic acid sequencing using the "Strand Sequencing" approach. Similarly, for protein 40 sensing other pores such as alpha-hemolysin (J Am Chem Soc., 2012, 134(5), 2781-7) and ClyA (Am. Chem. Soc. Nano. 2014, 8(12), 12826-35) (J. Am. Chem. Soc., 2013, 135(36), 13456-63) have also been adapted.

[0010] There remains a need for new nanopores that overcome the deficiencies of the prior art, not least in optimising the dimensions and characteristics of the pore for molecular sensing applications, and for example, nucleic acid sequencing applications.

[0011] Nanopore sensing is an approach to sensing that relies on the observation of individual binding or interaction events between analyte molecules and a receptor. Nanopore sensors can be created by placing a single pore of nanometer dimensions in an insulating membrane and measuring voltage-driven ionic transport through the pore in the presence of analyte molecules. The identity of an analyte is revealed through its distinctive current signature, notably the duration and extent of current block and the variance of current levels.

[0012] There is currently a need for rapid and cheap nucleic acid (e.g. DNA or RNA) sequencing technologies across a wide range of applications. Existing technologies are slow and expensive mainly because they rely on amplification techniques to produce large volumes of nucleic acid and require a high quantity of specialist fluorescent chemicals for signal detection. Nanopore sensing has the potential to provide rapid and cheap nucleic acid sequencing by reducing the quantity of nucleotide and reagents required.

[0013] Two of the essential components of sequencing nucleic acids using nanopore sensing are (1) the control of nucleic acid movement through the pore and (2) the discrimination of nucleotides as the nucleic acid polymer is moved through the pore. In the past, to achieve nucleotide discrimination the nucleic acid has been passed through a mutant of hemolysin. This has provided current signatures that have been shown to be sequence dependent. It has also been shown that a large number of nucleotides contribute to the observed current when a hemolysin pore is used, making a direct relationship between observed current and polynucleotide challenging.

[0014] While the current range for nucleotide discrimination has been improved through mutation of the hemolysin pore, a sequencing system would have higher performance if the current differences between nucleotides could be improved further. In addition, it has been observed that when the nucleic acids are moved through a pore, some current states show high variance. It has also been shown that some mutant hemolysin pores exhibit higher variance than others. While the variance of these states may contain sequence specific information, it is desirable to produce pores that have low variance to simplify the system. It is also desirable to reduce the number of nucleotides that contribute to the observed current.